Natriuretic effect of non-pressor doses of endothelin-1 in conscious dogs

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1. Renal, endocrine and haemodynamic responses to separate intravenous infusions of three doses of endothelin-1 (40, 400 and 4000 fmol kg^{-1} min^{-1}) were investigated in conscious dogs.

2. Administration of 40, 400 and 4000 fmol kg^{-1} min^{-1} endothelin-1 for 120 min increased plasma endothelin-1 levels by two-, seven- and 250-fold, respectively.

3. The two low doses did not have measurable effects on mean arterial blood pressure and heart rate but tended to increase glomerular filtration rate. The high dose increased mean arterial blood pressure (MABP; from 104 ± 4 to 138 ± 4 mmHg, $P < 0{\cdot}05$) and decreased heart rate (from 71 ± 4 to 46 ± 3 beats min^{-1}, $P < 0{\cdot}05$) as well as glomerular filtration rate (from 47 ± 3 to 19 ± 5 ml min^{-1}, $P < 0{\cdot}05$).

4. At rates of 40 and 400 fmol kg^{-1} min^{-1}, endothelin-1 increased sodium excretion about five- and eightfold, respectively. Relative changes in fractional sodium excretion were very similar. The high dose was markedly antinatriuretic (reducing sodium excretion from $8{\cdot}3 \pm 1{\cdot}1$ to $1{\cdot}2 \pm 0{\cdot}2$ μmol min^{-1}, $P < 0{\cdot}05$).

5. Diuresis increased during the administration of the two lower doses, which did not change plasma atrial natriuretic peptide or vasopressin concentrations. Urine flow increased after termination of the infusion of the pressor dose despite elevated plasma vasopressin and subnormal glomerular filtration rate.

6. Infusion of endothelin-1 at 40 fmol kg^{-1} min^{-1} did not change the concentrations of angiotensin II and atrial natriuretic peptide in plasma. Infusion of 400 fmol kg^{-1} min^{-1} was associated with a decrease in plasma angiotensin II, while plasma atrial natriuretic peptide was unchanged. The high dose of endothelin-1 markedly increased plasma levels of both hormones.

7. It is concluded that endothelin-1 at low plasma concentrations increases sodium excretion while a higher pressor dose of endothelin-1 is antinatriuretic. However, increases in plasma endothelin-1 seem to elicit diuresis over a wide concentration range, although possibly by different mechanisms.

Endothelins are the most potent vasoconstrictor peptides known (see review by Leppäluoto & Ruskoaho, 1992). Since the discovery of this peptide family, several studies have been performed to elucidate the possible participation of endothelin-1 (ET-1) in the regulation of fluid and electrolyte homeostasis. Available data on the renal effects of ET-1 provide an ambiguous picture. It has been shown in conscious dogs that relatively high doses of ET-1 decrease renal blood flow (RBF) (Goetz, Wang, Leadley, Zhu, Madwed & Bie, 1989) and glomerular filtration rate (GFR) (Emmeluth & Bie, 1992). Other results indicate that high doses of ET-1 constrict both afferent and efferent arterioles and decrease the glomerular ultrafiltration coefficient (Badr, Murray, Breyer, Takahashi, Inagami & Harris, 1989). Concomitant with the reduction in GFR, the rates of sodium and potassium excretion also decrease (Goetz, Wang, Madwed, Zhu & Leadley, 1988). At these high doses ET-1 also has systemic effects, such as increases in total peripheral resistance (TPR) and mean arterial blood pressure (MABP) (Goetz *et al.* 1989). As shown by Sørensen, Madsen & Pedersen (1994), the effects of ET-1 infusion in humans are similar to the effects seen in conscious dogs. However, in conscious rats it has been found that ET-1 may increase sodium excretion without affecting MABP (Garcia, Lachance & Thibault, 1990). These differences may be due to different experimental conditions, including dosage, or to species differences.

In anaesthetized rats, low doses of intravenous ET-1 have been shown to induce natriuresis while decreasing MABP (Harris, Zhuo, Mendelsohn & Skinner, 1991). It has also been reported that a low dose of ET-1 may decrease MABP and TPR in conscious dogs (Nakamoto *et al.* 1991), thus

indicating a possible vasodilator effect of continuous infusions in these species. Other results may hint at a more diverse spectrum of renal effects of ET-1. In our laboratory it has been found that the rate of urinary excretion of ET-1 increases when renal sodium excretion is augmented by a selective increase in the sodium concentration of the carotid blood (Emmeluth, Goetz, Drummer, Gerzer, Forssmann & Bie, 1994). Taken together, these findings suggest that intravenous ET-1 at low plasma concentrations may increase the rate of excretion of sodium.

Normal plasma ET-1 levels are about 4 pg ml^{-1} (molecular weight of endothelin-1, 2492; i.e. 1 pg $ml^{-1} \approx 0{\cdot}4$ pmol l^{-1}) in dogs (Emmeluth & Bie, 1992) and about 2 pg ml^{-1} in humans (Sørensen *et al.* 1994) and ET-1 seems to bind almost irreversibly to its receptors (Yanagisawa *et al.* 1988). Therefore, even small rates of infusion of ET-1 over prolonged periods of time may increase the concentration in plasma markedly. The renal effects of low doses of ET-1 (of the order of 10–100 fmol kg^{-1} min^{-1}) do not appear to have been investigated in conscious animals. Therefore, the present study was designed to test the hypothesis that ET-1 has a natriuretic effect at femtomolar doses in the conscious dog under precisely defined conditions with regard to body contents of sodium and water.

METHODS

Animals

Experiments were performed in six conscious female Beagle dogs weighing 8·5–15 kg. The dogs were fed a fixed diet of commercial dog food (Febo, Professional, Euskirchen, Germany) once a day around 14·00 h, and had free access to tap water. Daily sodium intake was 6–7 mmol kg^{-1}. Before the study, two surgical interventions were performed. After premedication (0·15 mg kg^{-1} propionylpromazine, 0·25 mg kg^{-1} methadone and 0·02 mg kg^{-1} atropine), general anaesthesia was initiated by a bolus of propofol (4 mg kg^{-1} I.V.) and maintained by continuous infusion of propofol (550 mg h^{-1}). The dogs were intubated and ventilated with a mixture of N_2O and O_2 (50 : 50) at a rate of 3·5 l min^{-1}. Using full antiseptic procedures, both common carotid arteries were displaced into skin loops to minimize the discomfort associated with subsequent arterial puncture. In addition, a chronic episiotomy was performed to facilitate catheterization of the bladder. All dogs were awake and ambulant 1 h after the infusion of propofol had been stopped and had completely recovered from the anaesthesia on the morning after the surgery. During the recovery and postsurgical period, the dogs were under veterinary inspection and treatment, including the administration of antibiotics for 6 days and analgesics when required. The dogs had no complications after surgery and were trained for several months before the experiments. Subsequently the animals were subjected to the following experimental procedures, which were approved by the Danish Animal Experiments Inspectorate.

Experimental protocol

The same six dogs were used for all experiments. In each dog experiments were performed at intervals of more than 1 week. At midnight, before the experiment, the water supply was interrupted by an electric valve controlled by a timer. On the experimental day, the dog was transferred to the laboratory at 08.00 h. A sterile catheter (Intracath, Becton Dickinson, UT, USA) was placed in a saphenous vein and used for blood sampling, another similar catheter was introduced to the right atrial area via an external jugular vein and used for infusion. A third catheter (Venflon, Viggo-Spectramed, Hälsingborg, Sweden) was placed in a common carotid artery to allow continuous measurements of arterial blood pressure, interrupted by the periodic sampling of arterial blood. The bladder was catheterized with a modified silicone Foley catheter (Norta, Beiersdorf AG, Hamburg, Germany). After the catheterizations, a 7·2 ml bolus injection of creatinine (13·6 mg kg^{-1}) was given, followed by continuous infusion of creatinine at a rate of 0·23 mg kg^{-1} min^{-1} for the rest of the experiment. During the experiment the body weight was kept constant by using an accurate servomechanism (Bie, 1976), which automatically and continuously replaces any loss in body weight with a hypotonic glucose–urea solution (40 mM glucose, 25 mM urea). The amounts of sodium excreted during the experiment were replaced by the following procedure. After the first urine sampling period, the urine sample was analysed and an infusion of a 200 mM NaCl solution was started and continued for the rest of the experiment. Calculations and adjustment of the infusion rate to match the excretion of sodium were made after each urine sampling based on urine analyses performed immediately after the sampling. Consequently, the excreted sodium was replaced with a delay of one urine sampling period (30 min).

The effects of ET-1 were examined by I.V. infusion of one dose on each experimental day. Three infusion experiments (40, 400 and 4000 fmol kg^{-1} min^{-1}) and one time control experiment were performed in each dog in a randomized order. Urine sampling was started ($t = 0$ min) 60 min after bolus injection of creatinine. Urine was sampled every 30 min. After a 30 min control period, the infusion of ET-1 or vehicle was started and continued for 120 min. After termination of the infusion, measurements were obtained for another 60 min (recovery). ET-1 was purchased from Peninsula Laboratories (Germany) and dissolved in the vehicle, which was hypotonic glucose–urea solution containing Haemaccel (Behringwerke AG, Marburg, Germany) (1 ml Haemaccel to 9 ml glucose–urea solution) to prevent adsorption to the utensils.

Blood samples were obtained 25 min into period 1 ($t = 25$ min), period 3 ($t = 85$ min), period 5 ($t = 145$ min) and period 7 ($t = 205$ min). A sample of venous blood (volume, 4 ml) was drawn from the saphenous catheter to allow the measurement of plasma sodium, potassium and creatinine concentrations and the osmolality. Also, 12 ml of arterial blood was drawn from the arterial catheter in order to measure arginine vasopressin (AVP), angiotensin II (ANGII), atrial natriuretic peptide (ANP) and endothelin-1 (ET-1) in plasma. Blood samples were centrifuged immediately at 4 °C and the plasma samples were stored at −18 °C.

Arterial blood pressure was measured by use of a pressure transducer (Statham P50, Gould) and a patient monitor (Dialogue 2000, Danica Elektronik, Rødovre, Denmark). The monitor continuously calculated mean arterial blood pressure (MABP) from the pressure signal, and heart rate (HR) from the electrocardiogram using a 300 Hz analog-to-digital sampling frequency and a 7 s time window. Calculated data were sampled from the monitor every 10 s by a computer and averaged over 30 min periods.

Analyses

Sodium and potassium ion concentrations in plasma and urine were measured by flame photometry using an IL243 LED flame

Table 1. Mean arterial blood pressure (MABP) and heart rate (HR)

		Endothelin-1 infusion					
Time (min)	0–30	30–60	60–90	90–120	120–150	150–180	180–210
MABP (mmHg)							
40	111 ± 3	110 ± 4	113 ± 5	111 ± 5	112 ± 4	113 ± 4	115 ± 5
400	109 ± 3	108 ± 4	113 ± 5	112 ± 3	112 ± 4	112 ± 3	114 ± 4
4000	104 ± 4	112 ± 5*	126 ± 5*†	135 ± 4*†	138 ± 4*†	131 ± 3*†	120 ± 4*
Vehicle	106 ± 3	105 ± 3	106 ± 3	109 ± 3	110 ± 3	110 ± 2	112 ± 3
HR (beats min^{-1})							
40	74 ± 4	71 ± 4	77 ± 6	74 ± 6	76 ± 6	81 ± 7	80 ± 7
400	73 ± 5	67 ± 4	70 ± 6	70 ± 5	69 ± 5	74 ± 5	79 ± 7
4000	71 ± 4	63 ± 4*†	49 ± 4*†	46 ± 3*†	46 ± 3*†	53 ± 4*†	58 ± 4*†
Vehicle	73 ± 6	72 ± 7	72 ± 2	70 ± 7	72 ± 6	72 ± 6	72 ± 6

Values are means ± S.E.M. The ET-1 dosage (in fmol kg^{-1} min^{-1}) is indicated by 40, 400 and 4000. *Significantly different from pre-infusion level ($P < 0.05$). †Significantly different from vehicle group ($P < 0.05$).

photometer (Instrumentation Laboratory, MA, USA). Osmolalities were measured by freezing-point depression (Advanced osmometer, model 3D3, Advanced Instruments, MA, USA). Creatinine was measured spectrophotometrically by a modified method of Jaffé described by Bonsnes & Taussky (1945).

The hormone analyses were performed by radioimmunoassay. First, the plasma and urine samples were acidified with 4% acetic acid and peptides were extracted by use of C-18 Sep-Pak cartridges (Waters, MA, USA), as previously described (Emmeluth & Bie, 1992). After elution, the samples were dried and stored at −18 °C in tubes topped with N_2. To determine the immunoreactivity of ET-1 in resuspended urine and plasma extracts, we used an antibody (RAS6901) purchased from Peninsula Laboratories and followed the procedure described by Emmeluth & Bie (1992). All urine samples were analysed in one assay and all plasma samples in another. The detection limit was <0·6 pg per tube and the extraction recovery of unlabelled ET-1 from urine was 90% and from plasma 100%. The intra-assay coefficient of variation (c.v.) in the urine and plasma assay runs were 14 and 10%, respectively, at an ET-1 concentration of 6 pg ml^{-1} in urine and plasma.

Immunoreactivity of AVP in plasma was determined by the technique described by Emmeluth *et al.* (1996) using an antibody (WAR) described earlier (Andersen, Andersen, Schütten, Warberg & Bie, 1990). The detection limit was <0·15 pg per tube. All samples were analysed in one assay and the extraction recovery of unlabelled AVP added to plasma was 85%. The intra-assay c.v. was 8% at an AVP concentration of 1·2 pg ml^{-1}. There was no cross-reactivity with oxytocin, vasotocin or angiotensin II. To determine immunoreactivity of ANGII in plasma, an antibody (Ab-5-030682) produced by Giese, Jørgensen, Nielsen, Lund & Munck (1970) was used. The procedure was described by Kappelgaard, Nielsen & Giese (1975) and the detection limit was 3 pg ml^{-1}. The samples were analysed in two assays and the extraction recovery of unlabelled ANGII was 85 and 93%, respectively. The intra-assay c.v. at an ANGII concentration of 40 pg ml^{-1} was 7%. Cross-reactivity with angiotensin I was 0·2%. Immunoreactivity of ANP in plasma was determined using an antibody (RAS8798) purchased from Peninsula Laboratories and the procedure described by Schütten, Johannessen, Torp-Pedersen, Sander-Jensen, Bie & Warberg (1987). The samples were analysed in two assays and the extraction recovery of unlabelled ANP was 70 and 80%, respectively. The intra-assay c.v. at an ANP concentration of 20 pg ml^{-1} was approximately 5%.

The results of ET-1, AVP, ANGII and ANP radioimmunoassays were not corrected for incomplete recovery.

Statistics

Data are presented as means ± S.E.M. The experimental results were evaluated by one-way analysis of variance (ANOVA) for repeated measurements. Bartlett's test was used to assess possible inhomogeneity of variance. When this was present, the data were transformed logarithmically before analysis of variance. When the results of the ANOVA were significant ($P < 0.05$) all differences between means were investigated systematically by Newman–Keuls test. P values smaller than 0·05 were considered to indicate significance.

RESULTS

Systemic haemodynamics

During the 2 h infusion of the two low doses of ET-1 no changes were observed in MABP and HR. The dose of 4000 fmol kg^{-1} min^{-1} increased MABP from 104 ± 4 to 138 ± 4 mmHg and decreased HR from 71 ± 4 to 46 ± 3 beats min^{-1} (Table 1). Notably, MABP decreased significantly during the first 30 min of the recovery period and HR showed a marked increase in the last 30 min of the recovery period. This indicates that the systemic haemodynamic effects of ET-1 subsided within 1 h. However, neither variable reached control levels within the recovery period.

Plasma osmolality and electrolytes

Plasma osmolality decreased slightly during the experiments in all of the four conditions (Table 2). The pattern and

degrees of changes were very similar in all of the conditions. Evidently, the procedure of concomitant replacement of fluid losses with hypotonic glucose–urea solution and salt losses by administration of 200 mM NaCl solution was unable to prevent a 1% decrease in plasma osmolality. However, significant changes in plasma sodium concentration were not detectable (Table 2).

Plasma potassium concentration did not change during infusion of the two low doses of ET-1 (Table 2). Administration of the high dose was associated with an increase in plasma potassium during the infusion. In the last period of recovery, plasma potassium decreased below pre-infusion levels.

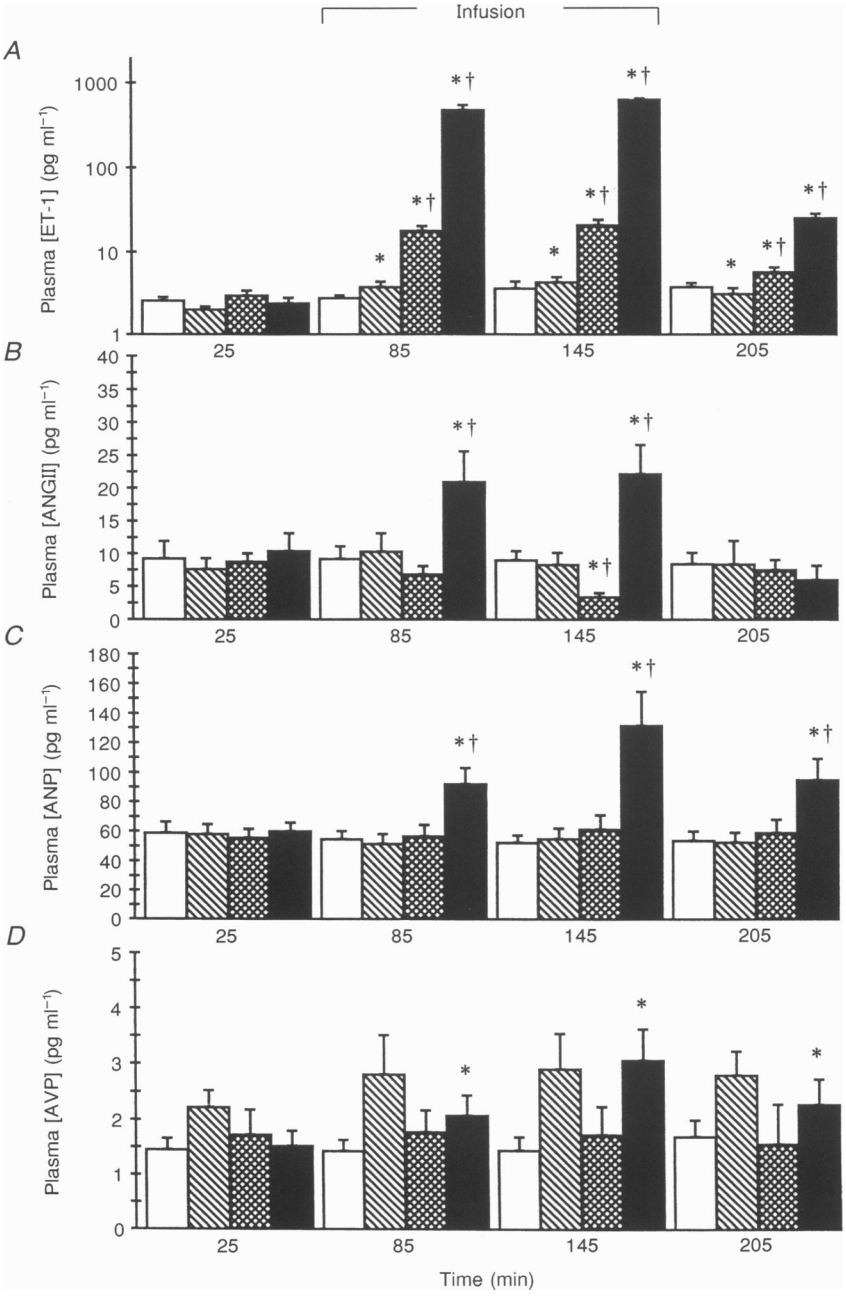

Figure 1. Plasma levels of endothelin-1 (ET-1), angiotensin II (ANGII), atrial natriuretic peptide (ANP) and vasopressin (AVP) during infusions of three different doses of endothelin-1

Doses: □, vehicle; ▨, 40; ▩, 400; ■, 4000 fmol kg^{-1} min^{-1}. Note the log scale for plasma ET-1 concentrations (*A*). The lowest dose of ET-1 did not change the plasma levels of ANGII (*B*), ANP (*C*) or AVP (*D*). The highest dose induced increases in all analysed plasma hormones, while 400 fmol kg^{-1} min^{-1} caused a small reduction in ANGII compared with the lowest dose. * Significantly different ($P < 0{\cdot}05$) from pre-infusion level. † Significantly different ($P < 0{\cdot}05$) from vehicle group.

Table 2. Plasma osmolality, plasma potassium and plasma sodium

		Endothelin-1 infusion		
Time (min)	25	85	145	205
Osmolality (mosmol kg^{-1})				
40	306 ± 1	305 ± 1*	303 ± 1*	303 ± 1*
400	305 ± 1	304 ± 1	303 ± 2*	303 ± 2*
4000	305 ± 1	304 ± 1	304 ± 1	304 ± 0·5
Vehicle	305 ± 1	303 ± 1*	302 ± 1*	301 ± 1*
Potassium (mmol l^{-1})				
40	3·9 ± 0·1	4·0 ± 0·1	3·8 ± 0·1	3·8 ± 0·1
400	3·7 ± 0·1	3·9 ± 0·1	3·8 ± 0·1	3·8 ± 0·1
4000	3·9 ± 0·1	4·1 ± 0·1*	4·1 ± 0·1*	3·7 ± 0·1*
Vehicle	4·0 ± 0·1	3·0 ± 0·05	3·9 ± 0·04	3·7 ± 0·03*
Sodium (mmol l^{-1})				
40	147 ± 1	146 ± 1	146 ± 0·4	146 ± 1
400	147 ± 1	147 ± 1	146 ± 1	145 ± 1
4000	147 ± 1	146 ± 1	145 ± 1	146 ± 1
Vehicle	146 ± 1	145 ± 1	145 ± 0·5	145 ± 1*

The ET-1 dosage (in fmol kg^{-1} min^{-1}) is indicated by 40, 400 and 4000. Values are means ± S.E.M.
* Significantly different from pre-infusion level ($P < 0·05$).

Hormones

Infusion of 40 fmol kg^{-1} min^{-1} increased the levels of plasma ET-1 about twofold compared with pre-infusion levels of 2·0 ± 0·2 pg ml^{-1} (Fig. 1). However, the elevated levels were not significantly different from the concentrations measured during infusion of vehicle. The lowest ET-1 dose did not change the concentrations in plasma of ANP, ANGII or AVP (Fig. 1). The dose of 400 fmol kg^{-1} min^{-1} increased plasma ET-1 about sevenfold (Fig. 1). This increase in plasma ET-1 was associated with a small but significant decrease in plasma ANGII. The concentrations of ANP and AVP in plasma were unchanged (Fig. 1). The infusion of ET-1 at a rate of 4000 fmol kg^{-1} min^{-1} increased the mean concentration of ET-1 in plasma by more than 250-fold (Fig. 1). Plasma ANP, ANGII and AVP concentrations all increased in response to this dose of ET-1 (Fig. 1).

Renal effects

Creatinine clearance was taken as a measure of glomerular filtration rate (GFR). Infusion of 40 fmol kg^{-1} min^{-1} ET-1 increased GFR compared with pre-infusion levels, whereas no significant change was measured in GFR during the infusion of 400 fmol kg^{-1} min^{-1} (Fig. 2). However, compared with the data obtained in the vehicle group (52 ± 3 ml min^{-1} at $t = 195$ min), GFR values of the 400 fmol kg^{-1} min^{-1} series were significantly elevated (57 ± 2 ml min^{-1} at $t = 195$ min, $P < 0·05$). Infusion of 4000 fmol kg^{-1} min^{-1} was associated with a marked reduction in GFR of 59% (Fig. 2). The decrease occurred gradually and was maximal after 90 min of infusion ($t = 120$ min). GFR did not return to control level within the 1 h recovery period.

Diuresis increased during infusions of 40 and 400 fmol kg^{-1} min^{-1} ET-1 (Fig. 2). These elevations of urine flow occurred without changes in MABP and plasma vasopressin concentration (Table 1, Fig. 1). During infusion of 4000 fmol kg^{-1} min^{-1} ET-1, diuresis remained unchanged despite a marked increase in MABP and plasma vasopressin concentration, possibly due to the 59% decrease in GFR. After termination of this infusion, MABP decreased towards, but not to, control values and diuresis increased even though GFR did not return to control levels.

The rate of urinary sodium excretion was constant in the vehicle group with mean values from 13 to 17 μmol min^{-1} (Fig. 3). After 1 h of infusion of 40 and 400 fmol kg^{-1} min^{-1} ET-1, sodium excretion was unchanged (Fig. 3). On the other hand, 1 h of infusion of 4000 fmol kg^{-1} min^{-1} significantly decreased sodium excretion and with this dose it remained reduced until the last 30 min of the recovery period. During the second hour of infusion of the two low doses of 40 and 400 fmol kg^{-1} min^{-1}, sodium excretion started to increase and this elevation reached maximal values in the recovery period. The doses of 40 and 400 fmol kg^{-1} min^{-1} increased mean sodium excretion five- and eightfold, respectively, with very similar time courses (Fig. 3).

Urinary potassium excretion did not change when the two low doses of ET-1 were infused (Table 3). During 4000 fmol kg^{-1} min^{-1} infusion, potassium excretion decreased but during the 60 min recovery period potassium excretion increased beyond pre-infusion levels (Table 3).

Since the dogs were slightly water deprived, the pre-infusion levels of urine osmolality were relatively high, i.e. 1200–1300 mosmol kg^{-1} (Table 3). Urine osmolality decreased modestly (< 20%) in the time control experiments. During administration of 400 fmol kg^{-1} min^{-1} ET-1, urine osmolality decreased, but not to levels significantly different from the vehicle group. In contrast, the high dose reduced urine osmolality to near isosmolar levels and thus increased

Table 3. Renal excretion parameters

		Endothelin-1 infusion					
Time (min)	0–30	30–60	60–90	90–120	120–150	150–180	180–210
Potassium excretion (μmol min^{-1})							
40	16 ± 1	17 ± 1	16 ± 2	18 ± 2	22 ± 7	23 ± 5	19 ± 3
400	16 ± 2	19 ± 2	18 ± 3	22 ± 3	20 ± 5	22 ± 4	22 ± 7
4000	16 ± 2	16 ± 2	8·1 ± 1·9*†	7·8 ± 2·3*†	11 ± 1	26 ± 2*	24 ± 1*
Vehicle	17 ± 2	20 ± 3	18 ± 2	21 ± 4	18 ± 3	19 ± 3	16 ± 2
Urine osmolality (mosmol kg^{-1})							
40	1232 ± 55	1168 ± 72	1133 ± 44	1121 ± 84	1028 ± 109	1013 ± 103	1054 ± 116
400	1280 ± 113	1251 ± 134	1116 ± 145	1075 ± 145	1041 ± 202*	940 ± 179*	789 ± 168*
4000	1185 ± 47	1130 ± 70	581 ± 90*†	331 ± 27*†	323 ± 8*†	369 ± 12*†	380 ± 43*†
Vehicle	1295 ± 99	1290 ± 87	1106 ± 67	1050 ± 69*	1111 ± 74	1123 ± 93	1020 ± 79*
Osmolar clearance (ml min^{-1})							
40	0·60 ± 0·05	0·61 ± 0·05	0·62 ± 0·05	0·75 ± 0·09	0·92 ± 0·22*	0·95 ± 0·17*	0·89 ± 0·07*
400	0·65 ± 0·06	0·68 ± 0·05	0·66 ± 0·05	0·80 ± 0·09	0·95 ± 0·17	1·02 ± 0·16*	1·05 ± 0·20*†
4000	0·67 ± 0·09	0·63 ± 0·10	0·33 ± 0·10*†	0·23 ± 0·09*†	0·24 ± 0·06*†	0·44 ± 0·04*†	0·59 ± 0·05
Vehicle	0·71 ± 0·03	0·74 ± 0·02	0·70 ± 0·02	0·76 ± 0·06	0·72 ± 0·04	0·71 ± 0·04	0·70 ± 0·03
Free water clearance (ml min^{-1})							
40	−0·45 ± 0·04	−0·45 ± 0·04	−0·45 ± 0·04	−0·53 ± 0·05	−0·57 ± 0·07	−0·62 ± 0·08	−0·62 ± 0·07
400	−0·49 ± 0·06	−0·51 ± 0·05	−0·46 ± 0·06	−0·55 ± 0·07	−0·58 ± 0·09	−0·57 ± 0·09	−0·52 ± 0·16
4000	−0·50 ± 0·07	−0·46 ± 0·08	−0·17 ± 0·07*†	−0·04 ± 0·03*†	−0·02 ± 0·01*†	−0·05 ± 0·03*†	−0·05 ± 0·07*†
Vehicle	−0·54 ± 0·03	−0·56 ± 0·03	−0·51 ± 0·02	−0·54 ± 0·04	−0·52 ± 0·03	−0·51 ± 0·04	−0·49 ± 0·04
Urinary ET-1 excretion (pg min^{-1})							
40	11 ± 2	—	10 ± 1	—	12 ± 4	—	9·0 ± 1·9
40	13 ± 2	—	8·8 ± 2·5	—	14 ± 3	—	16 ± 4
4000	8·6 ± 2·2	—	12 ± 8	—	11 ± 7	—	15 ± 7
Vehicle	12 ± 3	—	12 ± 2	—	12 ± 2	—	12 ± 2

Values are means ± S.E.M. The ET-1 dosage (in fmol kg^{-1} min^{-1}) is indicated by 40, 400 and 4000. * Significantly different from pre-infusion level ($P < 0·05$). † Significantly different from vehicle group ($P < 0·05$).

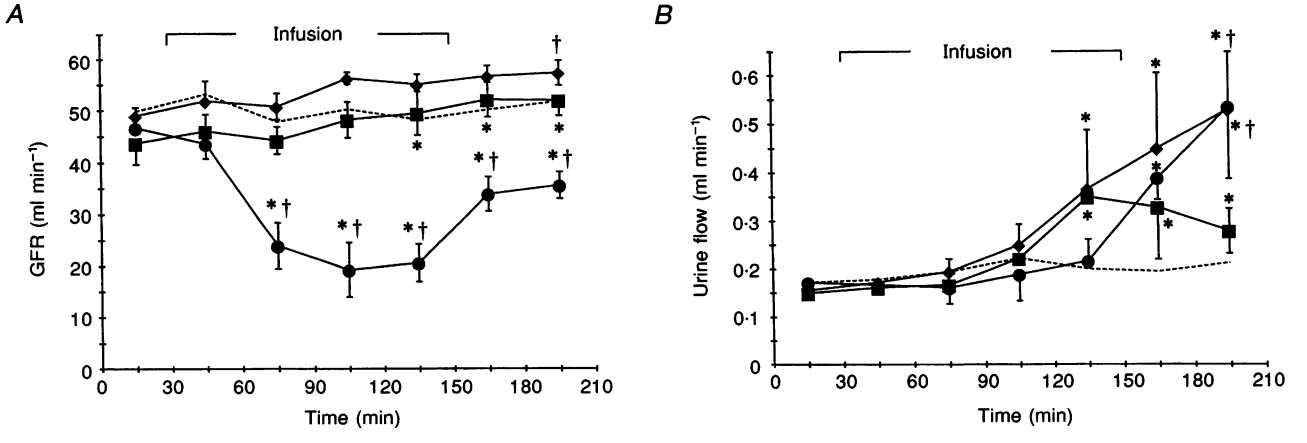

Figure 2. Glomerular filtration rate (GFR) and urine flow during infusions of endothelin-1

Doses: ■, 40; ◆, 400; ●, 4000 fmol kg^{-1} min^{-1}; and vehicle (dashed line). *A*, GFR; *B*, urine flow. The 2 low doses tended to increase GFR but neither of them was significantly different from both the pre-infusion level and vehicle group. The high dose elicited a marked decrease in GFR, which lasted for the rest of the experiment. The 3 doses of ET-1 increased urine flow but the increases in response to the 2 low doses were very variable. At $t = 105$ min the urine flow in the vehicle group made a small increase, which was significantly different from pre-infusion values. * Significantly different from pre-infusion level ($P < 0·05$). † Significantly different from vehicle group ($P < 0·05$).

free water clearance to values very close to zero ($-0{\cdot}02 \pm 0{\cdot}01$ ml min^{-1}). It is quite remarkable that this change occurred at a time ($t = 120$ min) when urine flow was not increased (Table 3).

Osmolar clearance increased during infusion of the two low doses of ET-1 (Table 3). Because urine flow showed similar increases with these doses, there were no changes in free water clearance (Table 3). The high dose decreased mean osmolar clearance by 66% and gradually increased urine flow more than threefold, which resulted in the mentioned increase in free water clearance.

Urinary ET-1 excretion remained unchanged during ET-1 infusions (Table 3). Remarkably, there was no association at all between the plasma level of ET-1 and the rate of excretion of ET-1.

DISCUSSION

In the present study we investigated the concomitant haemodynamic, endocrine and renal effects of three different doses of ET-1. One of the doses might be considered physiological because it doubled the concentration of ET-1 in plasma, one dose might be considered pathophysiological because it increased the plasma ET-1 level about sevenfold, i.e. to concentrations seen in various diseases, and one dose may appear pharmacological because it increased plasma ET-1 level about 250-fold. The results clearly demonstrate that small increases in plasma ET-1 enhance renal sodium excretion without measurably affecting systemic haemodynamics. Administration of the highest dose was associated with a substantial increase in MABP and marked decreases in HR, GFR, sodium and potassium excretion. The three doses all elicited diuresis.

The natriuretic effect of low doses of ET-1 is in accord with previous reports of experiments in anaesthetized rats (Harris *et al.* 1991). It cannot be excluded that the increase in sodium excretion found in the present study could be an effect secondary to an increase in GFR. In our study no or small increases occurred in GFR but fractional sodium excretion was enhanced about fivefold. The marked increase in fractional sodium excretion might suggest that ET-1 in low doses inhibits sodium reabsorption somewhere in the tubule. However, the increase in sodium excretion corresponds to an increase in GFR of only ~0·3 ml min^{-1} and the absolute rate of tubular sodium reabsorption, calculated as the difference between the rates at which sodium was filtered and excreted, did not decrease significantly in the experiments with the two low doses (results not shown). On the contrary, in one experimental series (40 fmol kg^{-1} min^{-1} ET-1) absolute sodium reabsorption rate increased slightly but significantly. In this series, therefore, the absolute rates of filtration, reabsorption and excretion of sodium all increased significantly, concomitant with the decrease in fractional reabsorption. These results emphasize the problem that small, and hardly detectable, increases in GFR may explain even a marked natriuresis. Therefore, the present data do not provide reliable information with regard to the mechanism of action of ET-1. Perico, Cornejo, Benigni, Malanchini, Ladny & Remuzzi (1991) reported that injection over 1 min of 150 pmol ET-1 increases sodium excretion more than twofold concomitant with an increase in lithium clearance and a decrease in GFR in the rat. Therefore, they suggested that ET-1 is an inhibitor of proximal tubular sodium and fluid reabsorption. Recently, this mechanism has been further elucidated in the isolated proximal straight tubule of the rat kidney (Garcia & Garvin, 1994). It was concluded that 10^{-9} M ET-1 added basolaterally

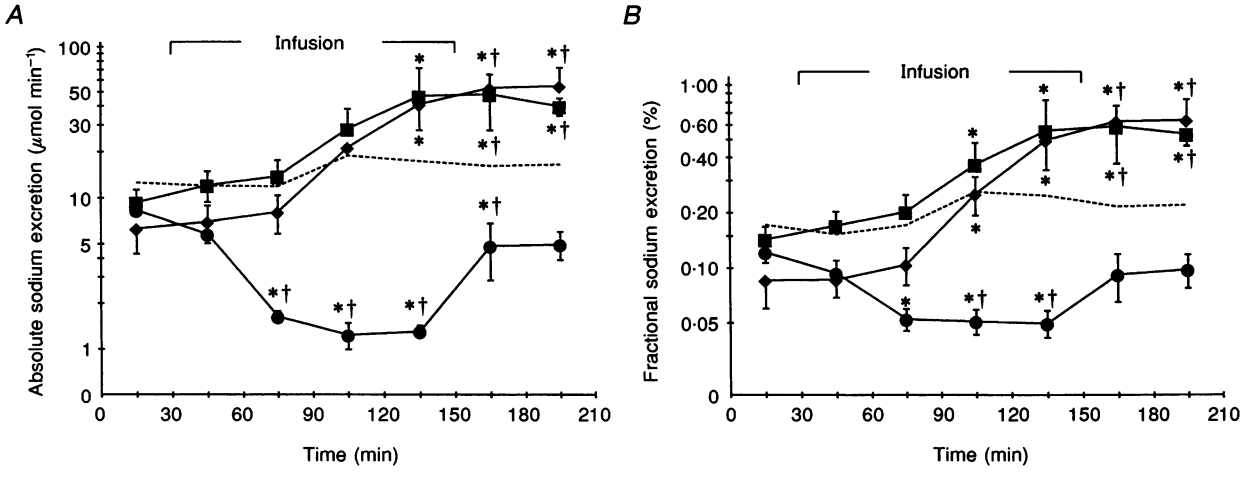

Figure 3. Sodium excretion and fractional sodium excretion during infusions of endothelin-1

Doses: ■, 40; ◆, 400; ●, 4000 fmol kg^{-1} min^{-1}; and vehicle (dashed line). *A*, absolute sodium excretion; *B*, fractional sodium excretion (both log scales). The 2 low doses of ET-1 increased both sodium excretion and fractional sodium excretion about fivefold. The high dose had a marked antinatriuretic effect. Remarkably, the curves for sodium excretion and fractional sodium excretion show a very similar time course. * Significantly different from pre-infusion level ($P < 0{\cdot}05$). † Significantly different from vehicle group ($P < 0{\cdot}05$).

inhibited tubular fluid reabsorption via activation of prostaglandin and leukotriene synthesis. Other data indicate that prostaglandins may be involved in the ET-1-mediated inhibition of Na^+–K^+-ATPase in inner medullary collecting duct cells (Zeidel, Brady, Kone, Gullan & Brenner, 1989). Consequently, these results are compatible with participation of arachidonic acid metabolites in the natriuretic effect of ET-1. However, further studies are needed to identify the renal processes involved in the natriuretic action of exogenous ET-1 in the intact animal.

ET-1 could induce a natriuresis by acting through other natriuretic hormones. The finding that ET-1 stimulates the secretion of ANP from cultured rat myocytes (Fukuda *et al.* 1988) suggests a mechanism in which the mediator could be ANP. In this study we saw no measurable increase in plasma ANP with the two low doses which did elicit clear-cut increases in sodium excretion. Therefore, circulating ANP does not seem to be the mediator of the observed five- to eightfold increase in sodium excretion. These results are similar to those obtained by Harris *et al.* (1991) who found that the natriuresis caused by exogenous ET-1 was not preceded by changes in plasma ANP.

ET-1 may inhibit the release of renin from rat juxtaglomerular cells (Takagi, Tsukada, Matsuoka & Yagi, 1989). It is thus pertinent to consider whether decreases in plasma ANGII and aldosterone could explain the natriuretic effect of low doses of ET-1. In the present study this seems unlikely because the concentration of ANGII in plasma did not change with administration of the lowest dose of ET-1. A small decrease did occur in plasma ANGII in response to 400 fmol kg^{-1} min^{-1} but the increase in sodium excretion in this series was not different from that seen with 40 fmol kg^{-1} min^{-1}.

The two low doses did not elicit measurable changes in either MABP or HR. In a previous study in conscious dogs (Nakamoto *et al.* 1991) it was found that 40 fmol kg^{-1} min^{-1} ET-1 produced a decrease – albeit a small one – in MABP and 400 fmol kg^{-1} min^{-1} increased MABP. Harris *et al.* (1991) also found a decrease in MABP in response to a low dose of ET-1 in anaesthetized rats. There is no obvious explanation for these discrepancies. It has been suggested that the natriuresis induced by ET-1 is a pressure-dependent event (King, Brenner & Anderson, 1989; Uzuner & Banks, 1993). In our study the natriuretic response to low doses of ET-1 could not be caused by an elevation of MABP because MABP did not change. In addition, when MABP was increased with the high dose of ET-1 antinatriuresis occurred.

ET-1 acts through at least two receptor subtypes, ET_A and ET_B (Kohan, Hughes & Perkins, 1992). These are unevenly distributed in the dog kidney (Brooks, DePalma, Pullen & Nambi, 1994) with ET_A to ET_B receptor ratios in cortical, medullary and papillary membranes of 22:78, 39:61 and 50:50, respectively. BQ123 (*cyc*(DTrp-DAsp-Pro-DVal-Leu) is assumed to be a specific ET_A receptor antagonist. A recent study of the response to ET-1 infusion in BQ123-treated anaesthetized dogs showed increases in sodium excretion, fractional sodium excretion and lithium clearance, and no renal haemodynamic effects (Clavell, Stingo, Margulies, Brandt & Burnett, 1995). This seems to exclude activation of ET_A receptors as the mechanism by which ET-1 induces natriuresis. It has also been reported that ET_B receptor activation with sarafotoxin S6c increases urine flow without any effect on absolute sodium excretion (Goetz *et al.* 1989; Brooks *et al.* 1994). This argues against ET_B receptors being mediators of the ET-1-induced natriuresis. If the above mentioned data are representative, the known effects mediated by ET_A and ET_B receptors do not appear to be sufficient to explain the present results.

The diuretic response to intravenous infusion of ET-1 is well known (Goetz *et al.* 1989; Nadler, Zimpelmann & Herbert, 1992; Schnermann, Lorenz, Briggs & Keiser, 1992) and does not seem to depend on the dose infused. The mechanism by which ET-1 increases urine flow has been investigated in several laboratories. *In vitro* studies show that ET-1 inhibits vasopressin-dependent cyclic adenosine monophosphate (cAMP) accumulation in the cortical, outer medullary and inner medullary collecting ducts (Tomita, Nonoguchi & Marumo, 1990), and thus inhibits the antidiuretic effect of vasopressin. These results are supported by the work of Nadler *et al.* (1992), who found that ET-1 inhibits vasopressin-stimulated water permeability in the inner medullary collecting duct. We found that small and moderate doses of ET-1 were not associated with significant deviations in the plasma concentration of vasopressin. The same doses clearly elicited a diuresis. The high dose of ET-1 was also diuretic in spite of an increase in plasma AVP. These results are fully compatible with an inhibitory effect of ET-1 on the renal action of vasopressin. It has been demonstrated that the collecting tubules express predominantly ET_B receptors (Terada, Tomita, Nonoguchi & Marumo, 1992; Takemoto, Uchida, Ogata & Kurokawa, 1993). Consequently, it seems most likely that ET-1 inhibits the effects of vasopressin through ET_B receptor activation (cf. Goetz *et al.* 1989; Brooks *et al.* 1994; Clavell *et al.* 1995).

The fact that renal tubular cell lines are capable of synthesizing endothelins (Kohan, 1991), together with the low plasma concentration occurring under normal conditions, has been taken as support for the notion that ET-1 acts mainly as a para- or autocrine mediator. The present results seem to indicate that circulating ET-1 may be a significant factor in the renal handling of sodium and water. A physiological dose that increased plasma ET-1 about twofold had obvious effects on sodium and water excretion without affecting the plasma levels of other relevant peptide hormones. On the other hand, infusions of ET-1 were not associated with increases in the rates of urinary ET-1 excretion (Table 2). This result is supported by the findings of Abassi, Tate, Golomb & Keiser (1992), who reported that

only 0·2% of an intravenous bolus of radiolabelled endothelin-1 appeared in the urine of rats. The relative independence between ET-1 concentrations in plasma and urine in the present experiment supports the notion that urinary endothelin is produced by the kidneys.

The high dose (4000 fmol kg^{-1} min^{-1}) increased the plasma ET-1 level by about 250-fold and had the expected potent vasoconstricting effect indicated by a substantial increase in MABP and a 35% decrease in HR. It is remarkable that a sevenfold increase in plasma ET-1 is not sufficient to cause peripheral vasoconstriction sufficient to change MABP or HR. Apparently vasoconstriction is not the predominant effect of plasma ET-1 at these levels. However, the present haemodynamic response to the high dose of ET-1 is in agreement with the findings of other high-dose experiments in conscious dogs (Goetz *et al.* 1988; Goetz *et al.* 1989; Emmeluth & Bie, 1992).

The high dose had an unexpected effect on plasma potassium concentration. During the infusion plasma potassium increased and during the recovery period plasma potassium decreased below pre-infusion values. This effect remains unexplained.

This study addressed cardiovascular and renal effects of circulating ET-1 at physiological, pathophysiological and pharmacological levels. Increasing concentrations of ET-1 seem to have a biphasic effect on sodium excretion. Femtomolar doses, which increase plasma ET-1 in the physiological and pathophysiological range, elicit natriuresis, while picomolar doses reduce sodium excretion. A similar dose–response relationship is not seen for the diuretic effect of ET-1. Urine flow increased in response to all doses although with apparently different time courses. The results indicate that plasma ET-1 may be an important modulating factor in the regulation of renal sodium and water excretion. ET-1 does not seem to exert its natriuretic and diuretic effects through changes in circulating ANP, ANGII or vasopressin levels. Furthermore, it is concluded that plasma ET-1 must be increased more than sevenfold in the normal dog to elicit a measurable elevation of MABP. Further studies are necessary to determine the sites of the renal actions of ET-1, and the receptor subtype involved in these actions.

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Acknowledgements

The authors thank Mrs Inge Pedersen, Mrs Birthe Lynderup, Mrs Barbara Sørensen, Mrs Karen Klausen and Mr Sigurd Kramer Hansen for technical assistance. The study was supported by The Danish Health Research Council and The Novo Foundation.

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Received 27 November 1995; accepted 23 April 1996.